# STUDIES ON SOME SOIL MICROBES IN RELATION TO ENVIRONMENTAL POLLUTION

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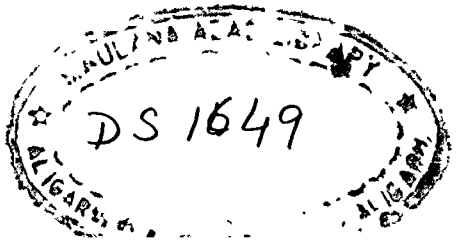
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Dedicated to
My Parents

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CERTIFICATE

This is to certify that Miss Kauser Jahan has prepared this dissertation as required for M.Phil (Botany) degree of the Aligarh Muslim University, Aligarh, under my supervision and guidance. She is allowed to submit this dissertation for evaluation in partial fulfilment of the requirements for the degree of M.Phil in Botany.

M. Wajid Khan
( Research Supervisor )

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# INTRODUCTION

Mycorrhizal fungi in symbiotic relationship with plant roots, help plants in acquiring mineral nutrients from the soil, especially immobile elements such as P, Zn, and Cu but also more mobile ions such as S, Ca, K, Fe, Mg, Mn, Cl, Br and N (Tinker, 1984). Mycorrhizal fungi also increase efficiency of mineral uptake, resulting in enhanced plant growth in soils where such elements are deficient or less available. Mycorrhizae also have been shown to increase water uptake and/or alter the plants physiology to reduce stress response to soil drought (Parke et al., 1983; Safir and Nelsen, 1985). They also reduce plant response to other soil stresses such as high salt levels; toxicities associated with mine spoils or land fills and heavy metals (Tinker, 1984). Mycorrhizal fungi in some cases reduce the disease response to plant pathogens causing some morphological or physiological changes in the plant (Dehne, 1982). Some mycorrhizal fungi produce metabolites that can alter the plants ability to produce roots from cuttings or to alter root regeneration and root morphology resulting in greatly increased absorptive surface area and feeder root longevity (Linderman and Call, 1977; Slankis, 1973). Mycorrhizal fungi are known also to alter soil texture by increasing the extent of soil particle aggregation (Sutton and Sheppard, 1976). Mycorrhizal fungi induce increased phytohormone production (i.e. cytokinins, gibberellins, and ethylene), playing a key role by influencing

and regulating the systems in plants (Allen et al., 1980; Barea and Azcon, 1982; Graham et al., 1981; Slankis, 1973). One of the major changes in mycorrhizal plants is reduced membrane permeability primarily due to increased P nutrition (Graham et al., 1981; Ratnayake et al., 1978). The decreased membrane permeability affects the quality and quantity of root exudation (Schwab et al., 1983) which in turn induce a significant response in the rhizosphere microflora and microfauna.

Mycorrhizae are divided into two groups-ectotrophic and endotrophic on the basis of structural differences (Harley, 1969). They are also differentiated into 5 main groups: ectotrophic mycorrhizae, vesicular-arbuscular, orchidaceous, ericoid and arbutoid mycorrhizae; the last four constitute the endotrophic mycorrhizae (Peyronel et al., 1969; Lewis, 1973). Vasicular-Arbuscular Mycorrhizae (VAM) are Endogonaceous fungi characterised by the production of both vesicles and arbuscules (Mosse, 1973; Callow et al., 1978), except the genus Gigaspora, which rarely produces vesicles in infected roots.

VAM fungi are found associated with most angiosperms, gymnosperms and ferns (Baylis, 1975; Trappe, 1977). Structures produced by VAM fungi within host roots include: a hyphal system contiguous, through initial penetration points, with a hyphal network extending into the soil; short-lived, intracellular arbuscules generally thought to function in nutrient transfer between the symbionts, and enlarged intercalary or

terminal vesicles that appear to function as endophytic storage organs (Carling and Brown, 1982).

The main effect of VA-mycorrhizae infection on plant growth is the stimulation of phosphorus uptake (Mosse, 1963, 1973; Gerdemann, 1976) due to exploration by the external hyphae of soil beyond the root hair and phosphorus depletion zones (Gray and Gerdemann, 1969). In addition, increased P uptake in mycorrhizal legumes stimulates nitrogen fixation by Rhizobium, thus indirectly causing an increase of N concentrations in the host (Carling et al., 1978; Schenk and Hinson, 1973). Changes in concentrations of other elements i.e. S, Zn, Cu, K and others in host tissues are known to be influenced by VA mycorrhizae (Gilmore, 1971; Gray and Gerdemann, 1973; Jackson et al., 1973; Lambert et al., 1979; Powell, 1975).

Legumes can form two types of symbiotic association with micro-organisms. One with nitrogen fixing species of Rhizobium and Bradyrhizobium, the other with vesicular-arbuscular mycorrhizal (VAM) fungi, concerned with the uptake of phosphorus by the plants. Glasshouse experiments have demonstrated that legumes inoculated with both types of micro-organisms grow and nodulate better and have higher nitrogenase activity and phosphorus content than plants that are uninoculated or inoculated with either root nodule bacteria or mycorrhizal fungi separately (Crush, 1974; Powell, 1976). The plants with both types of symbiosis may also be important as pioneer

colonisers of nutrient deficient habitats (Harley, 1973). Seed yield, shoot weight, and percentage of P and N of nodulated soybean plants grown in P-deficient field soil was increased by inoculation with Glomus fasciculatus (Bagyaraj et al., 1979). In related greenhouse studies on growth of soybeans, bean, alfalfa and peanut similar results were reported (Safir et al., 1972; Daft and El-Giahmi, 1975).

VA mycorrhizae may be influenced by a wide range of environmental and edaphic factors. Plant growth enhancement due to mycorrhizae was generally greater in sterile soil than in non-sterile soil (Gerdemann, 1968). The increased soil fertility decreases mycorrhizal development and plant growth response (Daft and Nicholson, 1972; Hayman and Mosse, 1972; Khan, 1975). Fewer spores were recovered from field soil of several agricultural crops when N or P fertility was optimal than when soils were nutrient deficient (Hayman, 1970; Hayman and Mosse, 1972).

Interactions between endomycorrhizal fungi and plant parasitic nematodes or soil-borne plant pathogenic fungi have also been reported. Mycorrhizal effects on nematode activities were usually more pronounced when plants were preinoculated with VAM fungi (Cooper and Grandison, 1986; Thompson et al., 1983; Smith, 1987). An increase in the level of host-resistance in mycorrhizal wheat to take-all disease was also attributed to P-nutrition (Graham and Menge, 1982).

Vesicular-arbuscular mycorrhizal (VAM) fungi are also affected by fungicides, pesticides, fumigants and herbicides. The growth of mycorrhizal plants were reduced by benomyl fungicide (Boatman et al., 1978). VA mycorrhizae alleviated the deleterious effect of the herbicide on plant growth when applied at moderate (0.05 mg/ml) but not at high (0.1 mg/ml) doses (Garcia Romera et al., 1988).

The symbiotic association of plants with microorganisms are affected by atmospheric pollution. In symbiotic or mutualistic association each component is beneficial to the other, and any adverse effect on one of the components will subsequently affect the entire system. Fumigation of seedlings of 'Troyer' citrange with ozone reduced mycorrhizae infection and chlamydospore production by Glomus fasciculatus (McCool et al., 1979).

Effect of gaseous air pollutants and simulated acid rain on roots and mycorrhizae has only recently received some attention. The ectomycorrhizae have been shown to alter some of the effects of air pollutants through promotion of shoot and root growth (Carney et al., 1978; Garrett et al., 1982; Mahoney et al., 1985). Red Oak (Quercus rubra L.) mycorrhizae were found to be adversely affected by ozone, sulphur dioxide and acidic precipitation (Reich et al., 1985). The vesicular-arbuscular mycorrhizal (VAM) fungi (endomycorrhizae) have received less attention in relation to gaseous and heavy metal environmental pollutants.

The toxic substances, responsible for pollution are termed as "pollutants". Environmental pollution is basically of three types viz., air pollution, water pollution and soil pollution. Wood (1968) classified air pollutants into two categories based on their origin i.e. primary and secondary air pollutants. Primary air pollutants are those that originate at the source in a form toxic to living organisms. Such air pollutants may be in gaseous or particulate forms. Gaseous air pollutants are sulphur dioxide ($SO_2$), oxides of nitrogen (NOx), hydrogen flouride (HF), ammonia ($NH_3$), ethylene ($C_2H_6$) etc. Particulate air pollutants are coal dust, cement dust, flyash, suspended particulate matter (SPM) etc. The secondary air pollutants are formed through reactions between primary air pollutants that originate from the source e.g. photochemical pollutants like peroxyacetyl nitrate (PAN) and ozone ($O_3$). $SO_2$ and NOx in high humid conditions are converted into acids ($H_2SO_4$ and $HNO_3$) which fall on the ground during atmospheric precipitation forming acid rain (Oden, 1968).

For the proper and healthy growth of the plants, certain, ranges of environmental factors like water, air, light, temperature, humidity, minerals etc. are necessary. Since over 90% biomass of green plants is derived from atmosphere and major parts of the plant body remain exposed to air, the quality of air is very important factor for health of the plant. Therefore, under polluted air there is real possibility of air-quality effects on plants. Air pollution adversely affects

plant life directly or indirectly and reduce the yield (Mudd and Kozlowski, 1975).

Gaseous air pollutants enter the leaves through stomata and cause injuries directly in the leaf tissue or interfere in biochemical reactions (Pell, 1979). Particulate air pollutants like soil dust, coal dust, cement dust, flyash etc. mostly fall and deposit on the leaf surface and block the stomatal cavities forming a thin encrustation on the leaf surface. This hampers transpiration and checks the transmission of solar radiation (Darley, 1966). The acid ($H_2SO_4$ and $HNO_3$) either directly injure the plant parts or indirectly through soil, harm the root system. Air pollutants affecting physiology and biochemistry of the plants, induce visible symptoms like chlorosis, necrosis, early senescence, stunting etc. (Heagle, 1973, 1982; Agrios, 1988).

Soil pollution due to heavy metals like Hg, Pb, Co, Cr, etc. through the discharge of industrial effluents and waste water has been recognised in many parts of the world. Refineries, metal smelters, caustic soda industries, paper mills, power plant discharges, soap factories, fertilizer industries, electroplating units etc. are mainly responsible for heavy metal pollution. Industrial effluents, domestic waste materials and sewage water are discharged into land and rivers which may reach to crop fields. The effluents and waste water containing a number of toxic substances when reach in the rhizosphere are absorbed by root and toxic substances accumulated in different

parts of the plant (Westing, 1969). It is believed that poor growth of the plants grown in such polluted soil might be due to reduce activity of the soil microflora which are beneficial to plant growth in addition to the direct effect of heavy metals on the root of the plant. The use of waste water for irrigation may raise serious problems for plants. Cole et al. (1969) stated that pollutants of this kind increase the prevalence of root disease and predispose the plants to pathogenic damage.

Acid rain influences species diversity and productivity in lakes and streams. The pH of soils, especially those with a low buffer capacity, may be decreased, and the leaching of exchangeable cations may be increased, by the addition of hydrogen ions from acid precipitation (Callow, et al., 1978). Such changes in soil chemistry may alter soil microbial populations or indirectly affect vegetative nutrition and productivity. Infection and telia formation by Cronartium fusiforme on leaves of willow oak seedlings were reduced by 'rain' of pH 3.2 (Shriner, 1974). This result was attributed to physiological changes in the leaves which reduced their susceptibility to infection. Generally fungi are less sensitive to acid conditions than are bacteria. Thus conditions which inhibit nodulation by Rhizobium may not affect colonization by mycorrhizal fungi. Rhizobium nodulation of kidney bean grown in a greenhouse or field plots (Shriner 1974) and soybeans grown in a greenhouse (Shriner, 1974; Waldron, 1978) was reduced by sulphuric acid rain of pH 3.2.

Plant sensitivity to $O_3$ is affected by plant nutrition. In a greenhouse, foliar injury of four soybean cultivars was greatest when a 6-25-15 NPK fertilizer was supplied at moderate than at low or high levels (Heagle et al., 1973). Some workers have reported reduced Rhizobium nodulation, nitrogen fixation, and/or leghaemoglobin content of legumenous plants following 1 or 2 acute $O_3$ exposures in greenhouse or controlled environment chambers (Blum and Heck, 1980; Blum and Tingey, 1977). In a greenhouse the number of Rhizobium nodules per plant and the nodule weight per plant of Dare soybeans were reduced 46 and 41% respectively by exposures to 0.25 ppm $O_3$ for 4 hr per day, 3 days per week, for 11 weeks (Reinert and Weber, 1980). Tingey (1978) suggested that a reduction in the amount of excess carbohydrates available for translocation to roots would cause less energy to be available for colonization by N-fixing bacteria. Root colonization by mycorrhizal fungi might be similarly affected by $O_3$.

In India, environmental pollution due to various kinds of industries, power plants, refineries and automobiles is quite common. These industries release $SO_2$, HF, NOx, $NH_3$, $O_3$, coal dust, cement dust, flyash in the form of air pollutants and industrial effluents and waste materials containing heavy metals in the form of soil pollutants. A number of crops are grown around the industries i.e. in the environment loaded with various kinds of pollutants.

The response of root nodule bacteria and vesicular-arbuscular mycorrhizae on plants growing under specific pollution stresses is the central theme of proposed work. Pulses, one of most important group of cultivated crops of India and major source of protein for a large vegetarian population in the country, will be selected as host crops for the proposed study. The plan of work would include only glasshouse experiments.

The system with components-host (pulses), symbiont-VAM fungi and nodule forming bacteria (Rhizobium) will be suitable model to ascertain the impact of environmental pollution. It is proposed to study the following aspects for Ph.D.

1. Effect of $SO_2$ on crop productivity of mungbean and chick-pea, root colonization by VAM fungi (Glomus sp. and Gigaspora sp.) and root nodulation (by Rhizobium).

2. Effect of $O_3$ on crop productivity of mungbean and chick-pea, root colonization by VAM fungi (Glomus sp. and Gigaspora sp.) and root nodulation.

3. Effect of $SO_2$ - $O_3$ on crop productivity of mungbean and chick-pea, root colonization by VAM fungi and root nodulation.

4. Effect of soil artificially amended with flyash on crop productivity of mungbean and chick-pea, root-colonization by VAM fungi and root nodulation.

5. Effect of treatments (1-4) on phosphorus and nitrogen contents of leaves, chlorophyll content of leaves and protein content of seeds.

# LITERATURE REVIEW

Mycorrhizal fungi are amongst the most common soil organisms in natural ecosystem. Several distinct symbiosis between fungi and roots of higher plants are grouped together as mycorrhizal associations. These associations have several features in common. The symbionts- the fungi and plant root maintain relatively constant association. Plant roots are normally infected by the fungi, and the mycorrhizal fungi are non-pathogenic. Disease symptoms are not apparent as a result of infection and growth of the higher plant is frequently enhanced by the development of mycorrhizal roots. On the basis of structural differences the mycorrhizal fungi are divided into two groups as Ectotrophic and Endotrophic mycorrhizae (Harley, 1969). Lewis (1975) in his modified classification divided all mycorrhizae into the following groups:

1. Ectotrophic mycorrhizae : Sheath; Hartig net; No intracellular penetration or lysis.

2. Vesicular-arbuscular mycorrhizae : No sheath; Extramatrical hyphae; Intracellular vesicles and arbuscules; Lysis.

3. Ericoid mycorrhizae : Sheath rare; Extramaterical hyphae; Intracellular coils; Lysis.

4. Arbutoid: Sheath; No hartig net; Intracellular haustoria; Lysis.

5. Orchidaceous : External hyphae; Intracellular coils; Lysis.

## Vesicular-Arbuscular Mycorrhizal (VAM) fungi

VA mycorrhizae occur in most angiosperms as well as in some gymnosperms, pteridophytes and bryophytes. VA mycorrhizae is absent only from a few plant families, mainly those which form only ectomycorrhiza (Pinaceae, Betulaceae) or the two specific types of endomycorrhiza (Ericales, Orchidaceae). Gerdemann (1968) listed 14 families which are believed to have little or no mycorrhizae including the Cruciferae (Brassicaceae) Chenopodiaceae, Caryophyllaceae, Polygonaceae, Juncaceae and Cyperaceae.

Vasicular-arbuscular mycorrhizal (VAM) fungi are obligate symbionts and characterised by intracellular infection within the root surface and bring about little or no change in external morphology of the infected roots. These fungi are classified in the family Endogonaceae of the order Endogonales in the class Zygomycetes. Gerdemann and Trappe (1974, 1975) have recognised the following five genera which form symbiotic association with plant roots.

1. Acaulospora 2. Endogone 3. Gigaspora
4. Glomus 5. Sclerocystis

Each genus has many species which are found in different hosts. Gigaspora and Acaulospora produce azygosporangia containing azygospores and are found free in soil. Glomus and Sclerocystis appear to be quite similar. Glomus is the larger of the two genera and contains forms that produce chlamydospores either free in soil or in sporocarps. The associations of these fungi with plant roots forming endotrophic mycorrhizae were first described by Janse (1897) and Gallaud (1905). Since then several other workers have described the VA mycorrhizal association as in the family Gramineae ( Poaceae), onion,bean roots, clover, rye grass, poinsettia, soybean, banana etc. (Nicolson 1959; Cox and Sanders, 1974; Holley & Peterson, 1979; Tisdal and Oades, 1979; Barrows and Roncadori, 1977; Schenck and Hinson, 1973; Iyer Rohini et al., 1988). Several hosts have also been examined ultra-structurally as yellow poplar (Kinden and Brown, 1975, 1976), bean (Holley and Peterson, 1979), onion (Gianinazzi et al., 1979).

Mycorrhizal fungi exist in soil as spores or as vegetative propagules in root fragments and VA infections are initiated following contact of a hypha originating from any one of these structures with a suitable host root and formation of an appresorium followed by penetration through or between epidermal cells, or by direct penetration without appresorium formation. All the three modes of penetration are observed in soybean. Penetration between epidermal cells following appresorium development is the most frequent means of ingress. After

penetration of the epidermis, fungal development is restricted to the root cortex. Inter-and/or intracellular hyphae develop from the point of penetration, at a greater rate longitudinally than in a radial or circumferential direction (Cox and Sanders 1974; Kinden and Brown, 1975). After penetration, arbuscules, highly branched, haustoria like structures, develop within the inner most cortical cells surrounding the cortical cylinder. They are considered to be the most probable sites of nutrient exchange between the symbionts - the fungus and the root. As arbuscular development begins, a dramatic increase in the volume of cytoplasm and host nuclei occurs with increasing maturity, the cytoplasm within the arbuscules becomes highly vacuolated and individual or portions of the branching system collapse. Deteriorating portion of arbuscular system aggregate into dense clumps. The period over which the arbuscules remain structurally and functionally intact is quite short, ranging from 4-15 days (Bevege and Bowen, 1975; Cox and Tinker, 1976).

Development of the mycorrhizal state involves little obvious change in root morphology. In some plants yellow pigmentation accompanies root colonization, and the endodermis may become thickened. Generally development of endomycorrhizae results in loss of root hairs, but no external fungal mental forms except for the relatively sparse external hyphae that occur at the rhizoplane and extend out into the soil.

Nutritional status of VA mycorrhizal plants is altered by activities of mycorrhizal root system. When available

phosphorus levels in the soil are low, VA infections stimulate significant increase in P uptake, resulting in dramatic increases in host growth (Gerdemann, 1968; Mosse, 1973). Changes in concentrations of other elements i.e. S, Zn, Cu, Sn and others are also known to be influenced by VA-mycorrhizae (Gilmore,1971; Gray and Gerdemann, 1973; Jackson et al., 1973; Lambert et al., 1979), but these changes are less dramatic than those in P. Hyphae extending into the rhizosphere from infected roots increase the effective P absorbing surface of the root by exploration of a larger volume of soil than is accessable to non-mycorrhizal roots. The additional surface area and the distribution of P-absorbing sites on the hyphae in the soil wholly account for the superior absorbing capabilities of VA mycorrhizal roots.

Translocation of absorbed P within hyphae to the host is quite rapid. Significant concentrations of $^{32}P$ in both root and leaf tissues of onion only 2 days after injection of the istope into soil 3-5 cm from mycorrhizal roots were recovered by Rhodes and Gerdemann (1975). Callow et al. (1978) proposed a scheme that absorbed phosphate is converted by the fungus to polyphosphate which is then translocated, as vacuolar granules, to components of the fungus within root tissues where it is subsequently degraded and made available for transfer to the host plant. It was also supported by Cox et al. (1975) and White and Brown (1979).

Mosse (1973) observed that the ability of VA fungi to colonize host roots is generally suppressed or eliminated if high levels of P are present in the soil. Studies of Menge et al. (1978) and Sanders (1975), however, showed that it is not P levels but rather concentrations of P within the host that cause the inhibition. Graham et al. (1981) stressed that alterations in root cell membranes and corresponding changes in root exudation, regulated by P levels in the host, is responsible for the P inhibition phenomenon.

The physiological changes that accompany the development of mycorrhizae, coupled with the chemical and physical impact of the fungal symbiont hyphae in surrounding soil, result in a very different potential in the rhizosphere. For this reason, Rambelli (1973) suggested the term "Mycorrhizosphere" used to describe the soil surrounding and influenced by mycorrhizae. Extramatrical hyphae that extend out some distance from the host tissues into the soil (Graham et al., 1982; Rhodes and Gerdemann, 1978) have a profound effect on the soil microflora. It has been shown that extramatrical hyphae of VA mycorrhizal fungi exude substances that cause soil and organic fractions to aggregate (Sutton, and Sheppard, 1976). Microorganisms flourish in the aggregates, and fungi, bacteria, actinomycetes, and algae (including cyanobacteria) have been isolated from them (Forster and Nicolson, 1981).

## Air pollution and its effects on plants

The atmosphere surrounding the urban and industrial regions of the world contains a mixture of chemicals commonly called as air pollutants. Many air pollutants are toxic to plants. The four most important of these in the order of their phytotoxicity are ozone ($O_3$), sulphur dioxide ($SO_2$), nitrogen dioxide ($NO_2$) and ammonia ($NH_3$). $O_3$ alone or in combination with $SO_2$ and/or $NO_2$, is responsible for upto 90% of the crop losses in some cases (Heck et al., 1982). Consequently, these phytotoxic air pollutants, alone or in mixtures, are of great concern to agricultural scientists. Air pollutants causing damage to plants have also been called plant pathogens (Wood, 1968). They injure plant foliage, significantly alter their growth and yield and change the quality of the marketable plant products. The air pollutants also increase or decrease the plant diseases caused by biotic plant pathogens (Heagle, 1973, 1982). Air pollution is analogous in many ways to the plant disease (Pell,1979). Broadly the air pollutants are of two types (Wood, 1968).

1. Primary pollutants
2. Secondary pollutants

Primary pollutants originate at the source in a form toxic to plants, e.g. $SO_2$, NOx, HF, $NH_3$, CO, $CO_2$, etc. Secondary pollutants are the result of reactions between pollutants originating from the source and other atmospheric factors e.g.

peroxyacetyl nitrate (PAN), $O_3$, acid rain, On the basis of their physical appearance, the air pollutants can also be grouped into two categories-gaseous and particulate. The most common gaseous air pollutants injurious to plants are $O_3$, PAN, $SO_2$, $Cl_2$, $C_2H_6$, HF, $H_2S$, NOx, etc. and the major particulate air pollutants are coal dust, flyash, cement dust, soil-dust particles etc. Some primary air pollutants i.e. NOx, $SO_2$ released into the atmosphere, when come in contact with the water and atmospheric precipitation are converted into the acids and fall down. This condition of environmental pollution is called as 'acid rain' (Likens and Borman, 1974). Acid rain is the most acute air pollution problem in developed countries while in developing countries acid rain problem is not so serious (Das, 1986). In India, 40-44% of air pollutants are particulate matters and these are extremely troublesome posing a great threat to plants and other living beings (Das, 1986).

The extent and nature of injury or damage caused by air pollutants is determined by genetic and environmental factors of plant as well as by level and duration of exposure to pollutants. The term 'injury' and 'damage' are often used interchangeably (Guderian et al., 1960). The living inducers of diseases are generally called as 'pathogens' by many pathologists. But diseases induced by abiotic factors e.g. air pollutants, drought, extremes of temperature etc. have many features in common with those induced by biotic pathogens. Cowling and Horsfall (1979), therefore, used the term 'pathogen' to denote

any inducer of disease. The pollutants affect physiology and biochemistry of plants resulting in the visible symptoms like chlorosis, necrosis, early senescence, stunting and several others abnormalities depending upon the type of air pollutant involved (Darley and Middleton, 1966; Brandt and Heck, 1968b; Barret and Benedict, 1970).

## Sulfur dioxide ($SO_2$)

Sulfur dioxide is one of the most important air pollutant and is pathogenic to plants. It is emitted mainly through the combustion of fossil fuels like coal and petroleum.

Its concentration at ground level depends upon the amount of emission, distance from the source and meteorological and topographical conditions. In general, $SO_2$ concentration decreases rapidly with distance from the source and with increased air movement. $SO_2$ concentration may be high as 1-3 ppm, near point sources, such as coal burning power plants and smelters. In large urban areas, $SO_2$ concentration may range from 0.05 - 0.40 ppm (Heagle, 1973).

$SO_2$ enters through the stomata in the mesophyll tissue of the leaves and reacts with water to produce sulphite ion which is slowly oxidized to sulphate ion. The sulphate ion may then be utilized by the plant as nutritional sulphur and converted to organic form (Thomas et al., 1944). The sulphite ions are, however, about 30 times more toxic than the sulphate ion (Thomas et al., 1943), when present in excessive amounts.

Chronic and acute markings appear depending upon the accumulation of sulphite ions (Barrett and Benedict, 1970). The chronic type of markings are general chlorotic appearance of the leaf, mild chlorosis, yellowing of leaf, silvering and bronzing of the undersurface. Acute injury resulting from the absorption of lethal quantities of $SO_2$ appears as marginal or intercostal areas of dead tissue. The areas at first show a grayish-green water-soaked appearance but on drying become bleached ivory in colour. The brown, red or black colours may be dominated. After a period of time, the dead or necrotic areas may fall out leaving a very ragged appearance on the leaf. When the major portion of the leaf is so injured an abscission layer often forms at the base of the petiole and leaf is shed (Barrett and Benedict, 1970). It causes chlorosis of leaves without formation of necrotic lesions and the veins characteristically remain green (Darley and Middleton, 1966; Agrios, 1988). Generally $SO_2$ reduces net photosynthesis in all plants at all concentrations but dark respiration and transpiration are increased.

$SO_2$ affects enzyme systems and metabolic processes in many plants. These changes are dependent on $SO_2$ concentration, plant species, plant age and environment. In some cases, enzyme activity is increased by exposure of the plants to low levels of $SO_2$ and decreased by higher concentrations (Horsman and Wellburn, 1977; Soldatini and Ziegler, 1979; Wyss and Brunold, 1980; Pierre and Queiroz, 1982; Tanaka et al., 1982). Plant metabolism is affected by $SO_2$ in a variety of ways. $SO_2$

stimulates phosphorus metabolism (Plesnicar, 1983) and reduces foliar chlorophyll concentration (Pandey and Rao, 1978; Lauenroth and Dodd, 1981). Carbohydrate levels are increased by low concentration of $SO_2$ and decreased by higher concentrations (Kozoil and Jordon, 1978).

Due to the effects of $SO_2$ on physiology and biochemistry of plant, growth, development and productivity are also affected significantly. The effects of $SO_2$ both in glasshouse and ambient air were studied by several workers. In the open top polythene chambers, wheat plants were exposed to 0.8 ppm $SO_2$ 2h daily for 60 days, although no chlorosis or necrosis in their leaves developed at any stage of their growth, reduction in root and shoot lengths, number and area of leaves per plant, biomass, productivity, number of grains per spike were reported (Pandey and Rao, 1978). In an exposure of 0.1 to 0.6 ppm $SO_2$ upto 100h, the wheat plant was less tolerant than maize and differences in sensitivities among cultivars were observed (Laurence, 1979). When the soybean was exposed to 0.09 to 0.79 ppm in a open air fumigation chamber, visible injury was not observed frequently while the reduction in the yield was found significantly (Sprugel et al., 1980). In groundnut, exposed to $SO_2$ ranging from 0.06 to 1.00 ppm for 4h daily for 6 weeks, the necrotic lesions and reduced net primary productivity were noticed at 0.25 ppm concentration and above. Below 0.25 ppm concentration, $SO_2$ was slightly beneficial to the plant productivity. Sulphur content of plant increased while nitrogen and phosphorus

contents decreased with increasing concentrations of $SO_2$ (Mishra, 1980). In alfalfa exposed continuously to 0.036 ppm concentration of $SO_2$, slight increase in biomass was reported (Lockyer and Cowling, 1981), while the biomass and yield of all the plant parts of snapbean were adversely effected above 0.1 ppm concentration of $SO_2$ for 12h per day till 5 days per week (Saxe, 1983b). Tomato plants exposed to 0.12 ppm concentration of $SO_2$ for 72h per week for 5 or 10 weeks showed slight decrease in ascorbic acid of ripe fruit but no effects were noticed on fruit yield and other soluble and total solid contents (Lotstein et al., 1983). Tobacco plants were found more tolerant to 0.02 ppm concentration of $SO_2$ than cucumber, treated continuously for 21 weeks. The dry weight of the plants was reduced, roots were more affected than the shoots and the flowering was also affected markedly (Mejstrick, 1980).

## Ozone ($O_3$)

Ozone is the most important plant pathogenic component of photochemical oxidant air pollution. Exhausts of automobiles and other internal combustion engines are probably the most important sources of ozone and other phytotoxic pollutants. Incompletely burned hydrocarbons and $NO_2$ are released into the atmosphere by the automobile exhaust. In the presence of UV light, this $NO_2$ reacts with oxygen and forms $O_3$ and NO. The ozone may react with NO to form the original compound. But in the presence of unburned hydrocarbons the NO reacts with

hydrocarbons instead of ozone and therefore, the $O_3$ is released in the atmosphere (Agrios, 1988). The naturally produced $O_3$ concentration at ground level is generally less than 0.03 ppm.

Ozone enters through stomata in leaves, where it accumulate in the palisade layer causing the bleaching or discoloration and the collapse of palisade cells. $O_3$ affects primarily expanding leaves, but not very young or old mature leaves. $O_3$ causes tippling, mottling and chlorosis of the leaf, usually on the upper leaf surface. The colour of the affected leaves varies from light tan to red or almost black, depending upon the plant affected. Affected leaves of some plants such as citrus, grapes and pines drop prematurely (Darley and Middleton, 1966; Agrios, 1988). Plant response to $O_3$ is, however, dependent on various environmental factors (Heck, 1968; Ting and Dugger, 1968).

The most common symptom on many deciduous trees, shrubs and some herbaceous plants is localized thickening and pigmentation of the cell walls resulting in sharply defined small dot-like coloured lesions (Ledbetter et al., 1959). Generally the interveinal region is injured, so lesions are usually angular in shape. The veins are usually not affected except in plants where pigment formation takes place. Pigment formation can produce on overall colouration of the upper leaf surface when the lesions are dense (Heck et al., 1970). Small unpigmented necrotic spots or more generally upper surface bleaching is a common type of injury on most of herbaceous and

many woody plants (Ledbetter et al., 1959). When the injury becomes more severe than upper epidermal cells collapse and become colourless. A shiny oily or waxy appearance of the upper leaf develops in some plants during the $O_3$ exposure. These symptoms disappear after the termination of the exposure. A water-soaked appearance often develops followed by drying and bleaching which results in typical bifacial necrosis within one or 2 days (Heck et al., 1970). Epidermal cells remain uninjured while the palisade cells and spongy mesophyll become injured. Many injured cells remain alive but chloroplast is disrupted and the chlorophyll amount is reduced significantly (Hill et al., 1961). Chlorotic mottling or chlorotic flecks are common symptoms on pine. Alfalfa develops large light green chlorotic areas with many irregular islands of normal green tissue dispersed in them. In some plants the tissue eventually becomes uniformly chlorotic and leaves may drop prematurely (Ledbetter et al., 1959).

Todd (1958) and Todd and Probst (1963) measured the effect of ozone at 4 ppm for 40 min. on photosynthesis and found that development of symptoms were associated with inhibition of $CO_2$ fixation. Hill and Littlefield (1969) noticed net photosynthesis decrease when plants were exposed to 0.06 ppm concentration of ozone for 1h. Pell and Brennan (1973) observed that in pinto bean net photosynthesis initially decreased and total adenylate concentration increased after a 3h exposure to injurious concentration of $O_3$. Net photosynthesis, however,

returned to normal within 24h. A significant increase in respiration of pinto bean leaves when exposed to 4 ppm for 40 min., was reported by Todd (1958). Mac Dowall (1965) found that during the first hour after ozone exposure at 0.7 ppm, the respiration was inhibited before the visible symptoms appeared; the respiration increased only later, when visible symptoms had appeared. Hill and Littlefield (1969) observed decrease in the rate of transpiration at 0.06 ppm concentration exposure for 1h. The sugar and starch content of the ash root decreased when exposed to 0.5 ppm concentration $O_3$ for 8h (Jensen, 1981). In a study Blum et al. (1982) found that $O_3$ at 0.1 ppm concentration increased the mineral content except the Na in ladino clover.

Ozone effects on the growth, biomass and productivity of several crops have been showed in both ambient as well as in glasshouse conditions (Heck et al., 1986). Ozone is reported to cause various types of damages in a number of crops like tomato, potato, pepper, sunflower, soybean, snapbean, clover etc. Ozone in the ambient condition was found to cause reduction in the tomato yield and responsible for 85% of reduced fruit size (Oshima et al., 1977a, 1977b). Tomato cv. Tiny-Tim when exposed to 0.08 - 0.10 ppm concentration of $O_3$ for 5h/day for 5 days in a week till 5 days, 56% reduction in fruit number and 91% reduction in fruit weight were observed (Manning and Feder, 1976). At the seedling stage, 0.4 ppm concentration exposure of $O_3$ for 2h, repeated for 6 time when transplanted in field, showed 57% reduction on the sensitive cultivars of

tomato (Henderson and Reinert, 1979). In the potato, ambient $O_3$ caused 60% leaf injury and 25% yield loss in cv. Norland and 31% loss in cv. Norchip. Cultivar Norland produced smaller tuber and cv. Norchip fewer tubers (Clarke et al., 1983). Ozone at 0.2 ppm concentration for 3h at several growth stages of potato in glasshouse decreased tuber weight and total solids but reducing sugars were increased (Pell et al., 1980). Ozone at 0.12 and 0.20 ppm concentration for 3h at 3 times per week reduced root, stem and leaf dry matter of pepper. The reduction was 16% at 0.12 ppm and 54% at 0.20 ppm in total fruit dry weight (Bennett et al., 1979). A decrease of 32% and 46% in root dry matters occurred in carrot cv. In cv. Acala SJ-2, a significant reduction in biomass and boll production was recorded, when exposed to 0.20 ppm concentration of $O_3$ for 6h twice a week. In one case this treatment started at the age of 8 day and other at 40 days age. Number of boll produced was reduce by 48% (Oshima et al., 1979). When sunflower was treated with 0.1 and 0.2 ppm concentration of $O_3$ for 12 days from the 14 days of age, 11% and 32% reduction in plant dry weight was observed. Due to $O_3$ exposure the roots were more affected than shoot (Shimizu et al., 1981). But when soybean exposed to 0.022 and 0.112 ppm concentration of $O_3$ for 7h/day in the field 39% reduction in yield and 12.6% reduction in oil content of the seeds were observed. Protein content of seeds, however, were not affected (Greenwald and Endress, 1984). When snapbean exposed to 0.30 and 0.60 ppm concentration of $O_3$ for 1.5h, two time at 6 different growth stages, reduction was found in the

growth rate and pod production (Blum and Heck, 1980). Reduction in the shoot and root growth of clover cv. Ladino was observed at 0.3 and 0.6 ppm concentration of $O_3$ treated for 2h at 4 different stages of growth (Letchworth and Blum, 1977). In a study when clover was exposed to 0.03, 0.06 and 0.09 ppm concentration of $O_3$ for 7h/day in the season for two years in the field condition, 14% and 27% reduction in forage regrowth at 0.06 and 0.09 ppm conditions respectively occurred during the second year (Blum et al., 1983a). At the 0.05, 0.10 and 0.15 ppm concentrations, the maximum root reduction (42%) and shoot reduction (24%) in the clover were observed when exposed for 4h/day for 6 days, 32 days after seedling (Blum et al.,1983b).

**Particulate air pollutants**

The major part of air pollutants are particulate matters. The major particulate air pollutants are coal dust, flyash, lime dust, cement dust, soil dust particles etc. Important sources of particulates are production of coal, cement, combustion of coal, gasoline and fuel oil, lime kiln operation; incineration and soil erosion, agricultural burning and wrong agricultural practices; volcanic erruptions, transportation and construction etc. In developing countries, particulate air pollutants are the major problem, and are not so important in developed countries (Das, 1986). In India 40-44% air pollutants are of particulate type.

Particulate matters settle on plant parts and cause

severe damage to the plants. They cause chlorosis, necrosis and death of the tissue, when the heavy deposition of the particles occurs. Many particles are by-products of agricultural practices and are usually inert (Darley and Middleton, 1966; Heck, et al., 1970).

Colwill et al. (1979) observed deposits on the leaves of the plants grown along the road-side with highly busy traffic. Such plants showed poor growth. There have been numerous reports that dust of varying origin interfere with stomatal functioning mostly by filling and blocking the stomatal aperture (Ricks and Williams, 1974; Fluckiger et al., 1978, 1979); increase leaf temperature (Eller, 1977; Fluckiger et al., 1978) and transpiration (Beasley, 1942; Eveling, 1969); reduce photosynthesis (Darley, 1966) and increase the uptake of gaseous air pollutants (Ricks and Williams, 1974). All these effects eventually results into poor growth of suffering plants.

**Effects of gaseous air pollutants and heavy metal pollutants on microorganisms**

Microbes and viruses are recipients of, and responders to atmospheric pollutants (Babich and Stotzky, 1978b). Atmospheric pollutants may adversely affect the generation time of bacteria, spore germination, mycelial proliferation, fruiting body formation and spore production by fungi, microbial respiratory activity, photosynthesis of cyanobacteria, algae and lichens;

nitrogenase activity of microbes involved in dinitrogen fixation, and viral infectivity. Furthermore microbial activities such as nitrification, denitrification, litter decomposition and mineralization of carbon, nitrogen and phosphorus and interactions such as host parasite, host saprophyte and mutualism are often adversely affected by atmospheric pollutants (Babich and Stotzky, 1974, 1978b).

Soil is a major sink for the removal of many gaseous pollutants. The removal of some pollutants by soil is reported e.g. sulphur dioxide, nitrogen dioxide, ozone and ammonia (Ferenbaugh et al., 1979; Abeles et al., 1971; Turner et al., 1973; Malo and Purvis, 1964). It appears to be by abiotic process. Microorganisms in soils may also be minor sink for the removal of atmospheric sulphur dioxide, as some fungi isolated from soil, e.g. species of Alternaria, Penicillium, Chaetomium, Colletotrichum, Trichoderma, Rhizopus and Fusarium oxysporum, were able to remove sulphur dioxide from the atmosphere (Cramer and Manning, 1974). The removal of atmospheric $SO_2$ and ammonia by soil is predominantly an abiotic process, once these gases come into contact with the soil solution, they form soluble products which may be utilized by the microbiota.

Microorganisms are also involved in another aspect of air pollution i.e. pollutant transformation. Industrial and demestic activities emit large quantities of non-gaseous,

inorganic pollutants, such as mercury, cadmium, lead, tin, selenium and arsenic. As a result various scavenging processes, these contaminants are deposited into aquatic and on to terrestrial environments, where one biochemical activity of microbes i.e. methylation, can change their chemical form. Methylation results in the conversion of inorganic volatiles and changes their potential ecological significance. Vonk and Sijpesteijn (1973); Hamdy and Noyes (1975) reported methylation of mercury in some fungi e.g. Aspergillus niger, Scopulariopsis sp. Saccharomyces cerevisiae and bacteria e.g. Psuedomonas spp., Mycobacterium spp. and Aerobacter spp.

The adverse influence of gaseous and heavy metal pollutants have been shown experimentally, as on the formation and germination and viral infectivity. Morphological abnormalities are also induced in microorganisms by the gaseous and heavy metal pollutants. The abnormalities may be permanent or temporary e.g. the conversion to subterranean hyphae during fumigation with ozone, and the reversion to aerial growth when ozone is removed (Hibben and Stotzky, 1969). Conidia of Alternaria solani germinate while still attached to conidiophore in the presence of ozone (Rich and Tomlinson, 1968).

The presence of microbes tolerant of heavy metals in environments contaminated with heavy metals is a common observation. Bacteria isolated from activated sewage sludge had high tolerances to heavy metals (Horitsu and Tomoyeda, 1975;

Table 1. Examples of adverse effects of some gaseous and heavy metal pollutants on the reproductive potential of microorganisms.

| Inhibited microrial response | Pollutant | Microorganisms |
|---|---|---|
| 1. Formation of fungal spores | Ozone | Botrytis cinerea (Krause and Weidensaul, 1978). |
| | Cadmium | Aspergillus niger, Trichoderma viride, Rhizopus stolonifer (Babich and Stotzky, 1977), Sclerotium rolfsii (Le Tourneau, 1978). |
| 2. Germination of fungal spores | Ozone | Verticillium albo-atrum, V. dahliae, Colletotrichum legenarium, Fusarium oxysporum, Botrytis allii, Trichoderma viride, Aspergillus niger, Penicillium egytiacum (Hibben and Stotzky, 1969). Botrytis cinerea (Krause and Weiden-saul 1978). |
| | Sulphur dioxide | Phytophthora infestans (Saunders, 1970), Diplocarpon rosae (Saunders, 1966). Alternaria sp. (Couey, 1965). |
| 3. Conjugation | Zinc | Escherichia coli (Ou and Anderson, 1972). |
| 4. Viral infectivity | Mercury | Bacteriophages of Staphylococcus aureus (Babich and Stotzky, 1979b) |
| | Peroxyace-tyl nitrate | Bacteriophage of Serratia marcescens (Peak and Belser, 1969). |

Horitsu et al., 1978); as heavy metal resistant bacteria, Bacillus sp. and Erwinia sp. (Austin et al., 1977). Numerous metal resistant fungi were isolated from soil contaminated by mine draining, and when cultured in laboratory media, Penicillium lilacinum ( Paecilomyces lilacinus) and Synnematium sp. tolerated 10,000 ppm cadmium whilst Penicillium waksmani and a Trichoderma sp. tolerated 8000 ppm cadmium (Tatsuyama et al., 1975).

The abiotic physico-chemical characteristics of the recipient environment influences the toxicity of pollutants to microbes in that environment (Babich and Stotzky, 1978a, 1979a, 1980; Stotzky and Babich, 1980). Some effects are summarized in Table 2.

Table 2. Examples of the influence of some abiotic environmental factors on the toxicity of some heavy metal pollutants to microorganisms.

| Abiotic factor | Pollutant | Microbial response |
|---|---|---|
| pH | Copper | The toxicity of Cu to spores and mycelium of Fusarium lycopersici decreased as the pH was decreased (Horsfall, 1956). |
| | Mercury | The toxicity of Hg to spores and mycelium of Fusarium lycopersici decreased as the pH was decreased (Horsfall, 1956). |
| | Cadmium | Penicillium asperum, Aspergillus niger, Cunninghamella sp. were more sensitive to Cd in an acidic soil than in alkaline soil (Babich and Stotzky, 1980). |
| | Lead | The toxicity of Pb to Trichoderma viride and Aspergillus niger increased as the pH was increased from acidic to alkaline levels (Babich and Stotzky, 1979a). |

Obligate fungal parasitism may also be retarded by exposure of the host plants to pollutants that damage the host tissue; for example, infectivity of oat by Puccinia coronata, of wheat by Puccinia graminis and of barley by Erysiphe graminis was reduced by exposures to ozone (Heagle, 1975) and the infectivity of Uromyces phaseoli on kidney bean was reduced by treatment with simulated acid rain. Blum and Tingey (1977) demonstrated that ozone affects root growth and nodulation of soybean through indirect effect on the foliage. Decreased photosynthesis, increased foliar respiration, and a retention of amino acids, proteins, and carbohydrates in foliage have been observed following $O_3$ exposures (Middleton, 1961; Tingey, 1978). The reduction in the amount of excess carbohydrate available for translocation to roots would cause less energy to be available for colonization by N-fixing bacteria. Root colonization by mycorrhizal fungi might be similarly affected by $O_3$ (Tingey, 1978).

**Interaction between mycorrhiza, soil-borne pathogens and other organisms:**

The mycorrhizal fungi may very well interact directly with other root pathogens, such as nematodes, because they show common and similar trophic requirements. Plant parasitic nematodes are obligate parasites of plants and some of them feed on the cortical tissues of roots. Therefore, potential for competition between exists for carbon and other nutrients. Such interactions result in reduction of infection or reproduction

of the nematode. The competition between plant pathogens and VA mycorrhizal fungi for the same host can influence the development of both, the pathogen and the mycorrhizal fungus. Priestel (1980) found that under the influence of Meloidogyne incognita, the colonization of cucumber roots by Glomus mosseae was adversely affected by the nematode. The nematode generally did not kill root cells. The negative influence on the mycorrhizal fungus was mediated by the host plant, as well as by the antagonism between endophyte and nematode. Damage to the root from the outside (e.g. by the action of toxins or by development of root rotting fungi in the cortex) will destroy the food base for the mycorrhizal fungi in the living root tissue (Davis, et al., 1978; Ross, 1972; Schenck and Kellam, 1978; Zambolin and Schenck, 1981). Increased plant disease resistance as a consequence of interactions between mycorrhizal fungi and plant pathogens is dependent on the biological influence of the endophyte.

Inoculation with VAM fungi or P pertilization increased host tolerance to nematodes except on tamarillo (Cooper and Grandison, 1987) and cotton (Smith et al., 1986) where P fertilization decreased host tolerance on P-fertilized, non-mycorrhizal tamarillo that was inoculated with M. incognita at planting but not when nematode inoculation was delayed 4 weeks.

Smith et al. (1986) repo ed that application of high levels of superphosphate to cotton grown in field microplots resulted in greater crop yield reduction due to M. incognita

than occurred in plots at a lower P levels or infested with VAM fungi. In studies on alfalfa (Grandison and Cooper, 1986), clover (Cooper and Grandison, 1986), cotton (Smith et al.,1986) and tomato (Cooper and Grandison 1986) nematode infection was generally greater on mycorrhizal and P-fertilized plants that had larger root systems compared to non-mycorrhizal plants grown at a lower P level. Nematode development, however, was always suppressed on mycorrhizal plants and increased on P-fertilized plants. Nematode damaged plants frequently show impaired water conductance through roots and deficiencies of B, N, Fe, Mg and Zn (Good, 1968). Thus VAM fungi may increase host tolerance by increasing uptake of key nutrients that would be deficient in a non-mycorrhizal nematode-infected plant.

The interactions between VA fungi and plant pathogens can be described in two general statements about mechanisms of resistance:

(Davis, 1980; Roncadori and Hussey,1977).

(i) Mycorrhizal fungi are able to retard pathogen development in the root system. This influence is restricted to the site of mycorrhizal establishment.

(ii) Mycorrhizal fungi are able to increase disease incidence systemically, especially in non-mycorrhizal plant parts. The systemic influence can be attributed to better nutrition, enhanced plant growth, and physiological stimulations in mycorrhizal plants. With increased concentrations of assimilates those plants can serve as better nutrient sources for plant parasitic organism.

There is a localized specific influence of VA mycorrhizal fungi on various alterations in host plant physiology. Mycorrhizal roots are more lignified than non-mycorrhizal ones, especially in stelar tissue (Dehne, 1982). This effect may be responsible for the restriction of the endophyte to the root cortex. The same mechanism of resistance may be effective against soil borne organisms invading the host root.

The induction of a higher resistance to root pathogens is limited to the biological interaction between host and endophyte. Increased resistance or decreased susceptibility requires optimum conditions for the development of the symbiosis, if possible before the attack of the pathogen. Pre-inoculation of tomato with a mycorrhizal fungus reduced the development of the plant parasitic nematode Rotylenchulus, nematode establishment with the formation of giant cells which seem to function as transfer cells for the nutrient flow towards the parasite (Sitaramaiah and Sikora, 1982).

## Effect of fumigants and fungicides on vesicular arbuscular (VA) mycorrhizal fungi

The fungicides and fumigants are used to control soil-borne pathogens. Most soil fumigants stimulate crop growth primarily because of the elimination of soil-borne pathogens. There have been consistent reports of stunting following fumigation with many crops including avocado (Martin et al., 1973), citrus (Kleinschmidt and Gerdemann, 1972; Martin et al., 1956;

O'Bannon and Nemec, 1978), cotton (Hurlimann, 1974; Wilhelm et al.,1967),peach (Lambert et al., 1979; La Rue et al., 1975), soybean (Ross and Harper, 1970), white clover (Powell, 1976), and hard wood tree species (Filer and Toole, 1968; Riffle, 1976; Bryan and Kormanik, 1977; Clark, 1963, 1969). Many fumigants have been reported to induce stunting by the fumigation including chloropicrin, D-D, ethylene bromide, methyl bromide etc. (Hurlimann, 1974; Martin, 1948; Martin et al., 1956; Filer and Toole, 1968) etc. Symptoms of the stunting syndrome include poor growth, and small, chlorotic leaves that may become necrotic at the edges. Older leaves abscise prematurely. Stems became thin and roots small but appear normal in the stunted condition (Lambart et al., 1979).

Kleinschmidt and Gerdemann (1972) identified the cause of the stunting following fumigation when they discovered that stunted citrus seedlings in a fumigated nursery lacked mycorrhizal fungi, whereas healthy seedlings were mycorrhizal. They also showed that both methyl bromide and steam could induce stunting of citrus by killing mycorrhizal fungi, while seedlings inoculated with mycorrhizal fungi grew normally.

Fungicides and fumigants may affect soil and rhizosphere populations of microorganisms. These microorganisms may interact with germination or infection by VA mycorrhizal fungi. Mosse (1962) showed that certain bacteria could significantly increase infection by infection of mycorrhizal fungi. Fumigants and fungicides may also reduce numbers of mycorrhizal hyperparasites

or predators which may increase mycorrhizal infection (Atilano and Van Gundy, 1979; Daniels and Menge, 1980).

Fungicides are generally far less damaging to mycorrhizal populations than fumigants. They typically delay or reduce VA mycorrhizal infection, but rarely eliminate it altogether. Some examples of the effects of fumigants upon root infection by VAM or development of chlamydospores of VAM fungi are summarized in Table 3.

Menge et al., 1978 determined the effect of different length of time and concentration of methyl bromide (MBr) on certain mycorrhizal fungi and found out that time of exposure decreased with the increase in conc. of MBr and vice-versa. The $LD^{90}$ (lethal dose) of MBr on chlamydospores of two mycorrhizal fungi was 12,000 ppm for 6 hr or more (C x T.72,000).

Mycorrhizal fungi are extremely sensitive to MBr. They are apparently twice as sensitive to MBr as Phytophthora parasitica and P. cinnamomi about four times more sensitive to MBr than Verticillium alboatrum and about nine times more sensitive to MBr than Sclerotium rolfsii (Munnecke et al., 1978).

Effect of some non-systemic fungicides upon root - infection or development of chlamydospores by vesicular-arbuscular (VA) mycorrhizal fungi are summarized in Table 4.

Table 3. Effect of fumigants on VAM fungi

| Fumigants | Host | Effect of fumigant on root infection by VAM fungi | Effect of fumigant on chlamydospore development by VAM | Reference |
|---|---|---|---|---|
| Chloropicrin | Cotton | reduced | reduced | Hurlimann (1974) |
| | Citrus | reduced | reduced | O'Bannon and Nemec (1978) |
| Methyl bromide | Yellow poplar | reduced | - | Clark (1963) |
| | Sweet gum | no effect to reduced | - | Filer and Toole, (1968) |
| | Pea | - | no effect | Stewart and Pfleger (1977) |
| | Sudan-grass | reduced | reduced | Menge et al., (1978) |
| | Citrus | reduced | - | Timmer and Leyden (1978) |
| 50% methyl bromide | Cotton | reduced | - | Wilhelm et al. (1967) |
| 50% chloropicrin | Cotton | reduced | - | Hurlimann (1974) |
| Ethylene dibromide | Citrus | no effect | - | O'Bannon and Nemec (1978) |
| | Citrus | increased to no effect | reduced to no effect | Nemec and O'Bannon (1979) |

Table 4. Effect of some non-systemic fungicides on VAM fungi

| Fungicide | Host | Effect of fungicides upon root infection by VAM | Effect of fungicide on chlamydospore development by VAM | Reference |
|---|---|---|---|---|
| Botran | Corn | reduced | - | Nesheim and Lin (1969) |
| Dicloran | Corn | reduced | - | El-Giahmi et al. (1976) |
| Captan | Corn | reduced | - | |
| | Wheat | no effect | no effect | Jalali and Domsch (1975) |
| | Bean | no effect to increased | - | Sutton and Sheppard (1976) |
| Copper sulphate | Pea | | no effect | Stewart and Pflegar (1977) |
| PCNB | Bean | reduced | - | Sutton and Sheppard (1976) |
| Sodium azide | Soybean | no effect | no effect | Kinloc and Schenek (1978) |
| | Citrus | no effect to increased | - | Nemec (1980) |

VAM fungi are also influenced by systemic fungicides. The effects of some systemic fungicides upon root infections or chlamydospore development by vesicular-arbuscular mycorrhizal fungi are given in Table 5.

Table 5. Effect of some systemic fungicides on VAM fungi

| Fungicide | Host | Effect of fungicide on root infection by VAM | Effect of fungicide on chlamy dospore development by VAM | Reference |
|---|---|---|---|---|
| Benomyl | Bean | reduced | - | Sutton and Sheppard (1976) |
| | Onion | reduced | - | DeBertoldi et al. (1977) |
| | soybean Red clover | reduced | - | Bailey and Safir (1978) |
| Calixin tridemorph | Wheat | reduced | reduced | Jalali and Domsch (1975) |
| Cela W524 triforme | Wheat | reduced | reduced | Jalali and Domsch (1975) |
| Thiabendazole | Wheat seed | reduced | - | Jalali and Domsch (1975) |
| | Bean | reduced | - | Sutton and Sheppard (1976) |
| Topsin (Thiophenate) | Wheat | no effect | reduced | Jalali and Domsch (1975) |

Graham et al. (1986) observed toxicity of fungicidal copper in soil to citrus seedlings and vesicular-arbuscular (VA) mycorrhizal fungi. Growth of seedlings and colonization by the mycorrhizal fungus (Glomus intraradices) were reduced logarithmically with Cu concentration. The Cu-induced P deficiency was attributed to inhibition of P uptake by mycorrhizal hyphae in soil.

Effect of gaseous and heavy metal pollutants om mycorrhiza

Plants are adversely affected by the pollutants. There have been many reports on the effects of gaseous air pollutants and simulated acid rain on conifer trees (Costonis, 1970; Wood and Borman, 1977). Obligate fungal parasites are also retarded by exposure of the host plants to pollutants that damage the host tissue. Infection of oat by Puccinia coronata; of wheat by Puccinia graminis; and of barley by Erysiphe graminis was reduced by exposures to ozone (Heagle, 1975), and infectivity of Uromyces phaseoli on kidney bean was reduced by treatment with simulated acid rain (Shriner, 1977).

Mycorrhiza symbiotic fungal root associations, are beneficial to the growth and development of plants (Beckjord et al., 1984; Mosse and Hayman, 1971). Some ectomycorrhizae are known to alter some of the effects of air pollutants through promotion of shoot and root growth (Garrett et al., 1982; Mahoney et al., 1985). Red oak (Quercus rubra L.) mycorrhizae were found to be adversely affected by ozone, sulphurdioxide and acidic precipitation (Reich et al., 1985). Keane and Manning (1988) observed interactions between $O_3$ and simulated acid rain (SAR) on ectomycorrhizae formation in birch seedlings. Interactions between $O_3$ and SAR, SAR and mycorrhizal treatment, soil regime and mycorrhizal treatment and ozone and soil regime had significant effects. Treatment of seedlings with pH 3.5 SAR caused increases in growth which were more apparent in birch exposed to $O_3$. Mycorrhizal treatment caused increased

growth in non-steamed soil, while growth decreased in steamed soil. Birch seedlings grew much better in steamed soil.

The effect of acid rain and ozone on soybean plant with endomycorrhizal fungi Glomus macrocarpus was observed by Feicht (1981). The simulated acid rain of pH 3.2 or 2.8 did not affect percentage root colonization by G. macrocarpus (Feicht, 1981), and ozone reduced spore production of G. macrocarpus without reducing colonization. Since $O_3$ does not penetrate the soil directly, the effect of $O_3$ on the fungus was probably mediated through an effect on host metabolism. The total number of chlamydospores per unit dry weight of root was reduced by 0.08 ppm $O_3$. The effect of $O_3$ on the fungus was greater than the effect on the host alone (Blum and Tingey, 1977). Mc Cool et al. (1979) found that weekly acute $O_3$ exposures eliminated the beneficial growth response for troyer citrange orange seedlings to infection by G. fasciculatus. It was suggested that $O_3$ altered host metabolism by changing percent P, K, Ca, and Na in citrange leaves and thus affected the mycorrhizal symbiosis and reduced percentage root infection.

Vesicular-arbuscular (VA) mycorrhizal fungi can stimulate plant metal uptake in soils where the metals are sparingly available. Tinker (1984), suggested that the influence of VAM fungi on metal uptake should be studied when the host plants are growing in soils containing potentially toxic levels of heavy metals. Such environments might include soils in the

vicinity of metal mines, metalliferrous bedrock or soils down wind of metal smelters, since smelter effluents result in both acidic and heavy metal depositions and biological availability of heavy metals generally increases with acidity.

The growth, survival and heavy metal content of two races of calluna, one from a metal-polluted site and one from an unpolluted natural heath-land, have been compared when plants were grown in the mycorrhizal (M) and non-mycorrhizal (NM) condition in sand cultures supplemented with different levels of copper and zinc. The NM plants showed no tolerance of these metals at high concentrations; mycorrhizal infection provided a major degree of resistance to the toxicity and infection led to significant reduction of the heavy metal content of shoot (Bradley et al., 1981). Gildon and Tinker (1983a) reported that the degree of infection of onions with the vesicular-arbuscular mycorrhizal fungus, Glomus mosseae was strongly reduced by additions of zinc, copper, nickel or cadmium to the soil medium. A split pot experiment was used to show that zinc translocated within the plant from other roots was effective in decreasing infection levels. It was also observed by Gildon and Tinker (1983b) that clover plants growing on areas which had been heavily contaminated with metal were found to be strongly infected with mycorrhizal fungi. A comparison of G. mosseae isolated from these plants with the isolate used at Rothmsted showed the former to be much more tolerant of zinc and cadmium in the soil. There was some indication that mycorrhizal infection, particularly with the tolerant isolate,

could protect plants against the effects of heavy metal additions. It was also reported that an infection with mycorrhizal fungi can increase the supply of copper to the host plant, in conditions where increased phosphorus nutrition does not increase dry weight. It is suggested that copper is absorbed and translocated by mycorrhizal hyphae in a manner analogous to that which occurs for phosphorus (Gildon and Tinker, 1983b). Dixon and Buchsena (1988) reported the response of ectomycorrhizal Pinus banksiana and Picea glauca to heavy metals in soils. Ectomycorrhizal colonization rates were sufficiently reduced on Pinus and Picea seedlings by the heavy metals particularly Cd and Ni. Needle tissue metal concentrations were lower in ectomycorrhizal seedlings at low soil metal concentrations. However, at higher soil concentrations, heavy metal concentrations of needle tissue were similar in ectomycorrhizal and non-mycorrhizal plants. The growth of non-mycorrhizal seedlings exposed to heavy metals was reduced compared to those inoculated with Suillus luteus, apparently ectomycorrhizal colonization can protected Pinus and Picea seedlings from heavy metal toxicity at low or intermediate soil concentrations of Cd, Cu, Ni, Pb, and Zn.

## ROOT-NODULE BACTERIA

Root-nodule bacteria fix the atmospheric nitrogen symbiotically in association with leguminous plants. The nodule forming bacteria are the species of Rhizobium and Bradyrhizobium

in the family Rhizobiaceae. The nodule forming bacteria are gram negative rodshaped of short to medium size,and live freely in soil and in the root region of both leguminous and non-leguminous plants. They enter into symbiosis only with leguminous plants, by infecting their roots and forming nodules on them. They show specificity in their symbiotic association with legumes. Therefore, there are specific strains for a legume or a group of legumes. The nodule forming bacteria are recognized as microsymbionts. When nodule becomes senescent after a period of nitrogen fixation, decay of tissue sets in liberating motile forms of bacteria into soil which normally serve as a source of inoculum for the succeeding crop of a given species of legume (Rao, 1972, 1975). The nodule forming bacteria penetrate the root from the root hair through the intercellular spaces and form nodule in the upper cortical regions. The core of a mature nodule constitutes the bacteriod zone surrounded by several layers of cortical cells. The volume of bacteroid zone in effective nodules has a direct positive relationship with the nitrogen fixed. The effective nodules are generally large and pink in colour due to leghaemoglobin (Bergersen and Briggs, 1958).

The nodulated legume system depends only on the available mineral nitrogen in the soil. A close relationship is established between host symbiont and the environment. The effectiveness and efficiency of the Rhizobium - legume symbiosis are dependent on this relationship.

The factors limiting the bacteria include the ability to compete, their capacity to survive, and hydrogenase activity. The factors limiting the host include rates of photosynthesis and nutrient uptake. Environmental factors include the P, K, Ca, N, micronutrient and moisture.

Soil acidity influence $N_2$ fixation by direct and indirect effects onthe bacteria and on the host. Rhizobium species and strains vary in their tolerance to soil pH. Munns (1976) and Munns et al. (1977) reported that the behaviour of one legume in acid soil cannot be deduced from experiencing with another i.e. among the tropical and temperate legumes, one can find both tolerant and intolerant species and hence different responses to lime.

Freire (1976) reviewed the effects of soil acidity and pH related factors on soybean nodulation and $N_2$ fixation. Growth and activity of Rhizobium as well as effective nodulation requires an adequate supply of Ca. There is some correlation between soil acidity and the availability to plants of such macro-and micronutrients as Ca, P, Mn, Al, B, Mo and Cu. Lime applications correct Al and Mn, and it also reduces or increases the availability of many other elements and nutrients in soil.

Graham and Halliday (1976) reported that soil temperature is a major limiting factor for beans in tropical and subtropical areas, the limiting pH for growth of R. phaseoli in liquid medium was 4.0 to 4.4.

In some soils, P fertilization by itself alleviates the inhibition of nodulation, but where toxic levels of Al and/or Mn are present, liming is essential to promote adequate nodulation for high $N_2$-fixation and high yield. The direct effect of Al and Mn toxicities on Rhizobia was studied by Keyser and Munns (1979). They reported that in acid soils Al-toxicity and acidity itself were probably more important factors limiting Rhizobial growth than Mn toxicity and Ca deficiency. Potassium, S and micronutrients in soil have minor roles as limiting factors, at least in areas of low productivity agriculture (Andrew, 1976; Freire, 1976; Franco, 1976).

Diatloff (1967) observed that during wet periods in a black earth soil, aeration was the limiting factor for nodulation of cowpea, soybeans, and native legumes. Goepfert and Freire (1973) obtained significant increases in nodulation and dry matter of Phaseolus vulgaris in a soil sieved to obtain particles of 0.8 to 2.0 mm.

It has been shown by Franco (1976) that plants dependent on $N_2$ require more P than plants using mineral N. P deficiency is the most important single limiting factor for $N_2$ fixation and legume production. There is the vital role of P in energy transfer and large quantity of energy required for the reduction of $N_2$ to $NH_3$. The addition of P to soil usually causes an increase in the concentrations of P and N in plant tissues. This relationship has been shown for tropical pasture legumes (Andrew, 1976). According to Andrew (1976) the increase in

N-concentration in pasture legumes resulting from P additions may be compounded by time and energy content of nodulation and by duration and efficiency of symbiosis. Goepfert (1971) reported a close relationship in soybeans between grain yield, P availability in the soil, and nodule weight of soybeans.

Mosse (1976) believed that the effect of vesicular-Arbuscular-mycorrhizae (VAM) in improving phosphate supply to plants results from the absorbing capacity of the extensive network of external hyphae associated with the infected roots. Nodulation of various legume species has been shown to be responsive to the inoculation with mycorrhizae.

The reduced Rhizobium nodulation, nitrogen fixation, and/or leghemoglobin content were observed in leguminous plants following 1 or 2 acute $O_3$ exposures in greenhouse or controlled environment chambers (Blum and Heck, 1980; Blum and Tingey, 1977). In a green-house, the number of Rhizobium nodules per plant and the nodule weight per plant of soybean were reduced 46 and 41% respectively, by exposures to 0.25 ppm $O_3$ for 4 hr per day, 3 days per week, for 11 weeks (Reinert and Weber, 1980).

Rhizobium nodulation of kidney beans grown in a greenhouse or field plots and soybeans grown in a greenhouse (Shriner, 1974; Waldron, 1978) was reduced by sulphuric acid rain of pH 3.2. Mc Guirt (1976) reported that nodulation of soybeans grown in field-plots was not reduced by rain of pH 3.5. Waldran (1978)

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separated the effects of $H^+$ and $SO_4^-$ ions on nodulation by 'rain' or soil drench applications of sulphuric acid (pH 3.2) or sodium sulfate (pH 5.7). He found that $H^+$ ions were the major cause of the inhibition, however, $SO_4^-$ ions caused a slight reduction in nodulation when applied as 'rain' Shriner (1974) and Waldron (1978) suggested that a substance leached from the foliage may also have contributed to the inhibition of nodulation.

**Interaction between VAM fungi and root-nodule bacteria**

Significant interactions between selected bacteria and VA mycorrhizal fungi, have been recorded. Dual inoculations with VA mycorrhizal fungi and rhizobacteria have resulted in increased mycorrhizal colonization (Bagyaraj and Menge, 1978). The microbial interactions in the mycorrhizosphere of VA mycorrhizal have been reviewed by Barea and Azcon-Aguilar (1982) and Bagyaraj (1984). Bagyaraj and Menge (1978) reported an increase in rhizosphere populations of bacteria and actinomycetes when plants were inoculated with Azotobactor or VA mycorrhiza, inoculated singly or in combination but more from the combination. The combined inoculation resulted in synergistic growth enhancement of the host plant. Meyer and Linderman (1986) compared rhizoplane and rhizosphere soil in regard to the selection of qualitatively different functional groups of bacteria from the naturally occuring microflora. They showed no quantitative difference in total bacteria in the rhizosphere

soils from mycorrhizal plants, but significant qualitative shifts were found. For example, facultative anaerobes (possible nitrogen fixers and ethylene producers) increased in the mycorrhizosphere soil, but flourescent Psuedomonads decreased.

Inoculation of legumes with VA mycorrhizal fungi and Rhizobium in some soils was found to have synergistic beneficial effects on nodulation, nitrogen fixation and legume growth (Carling et al., 1978; Daft and El-Giahmi, 1974; Islam et al., 1980). Legumes require adequate phosphorus supply for satisfactory nodule production and nitrogen fixation (Mosse, 1977; Van Schreven, 1958). Inoculation of soybean with Glomus fasciculatus and Rhizobium japonicum significantly increased dry weight and nitrogen content of the shoot and total dry weight, nodule dry weight, nitrogenase and nitrate reductase activities (Bagyaraj et al., 1979; Carling et al., 1978) as compared to inoculations with either of the organism alone. Inoculation of Leucaena leucocephala with G. fasciculatus and Rhizobium in a P-deficient soil improved nodulation, mycorrhizal colonization, dry weight and N and P contents of the plants compared to single inoculation with either organisms (Manjunath et al., 1984). The improvement of P uptake by the host plant resulting from VAM infection enhances nodulation and N-fixation.

Effectiveness among four VA mycorrhizal fungi and Rhizobium in promoting growth of three legume trees in P-deficient soil was studied by Delacruz et al. (1988).

Glomus fasciculatus with Rhizobium, and Gigaspora margarita with Rhizobium were most effective for Acacia mangium and Albizzia falcataria (Syn: Paraserianthes falcataria). Scutellospora persica with Rhizobium, Gigaspora margarita with Rhizobium and Glomus fasciculatus and Rhizobium were most effective for Acacia auriculifornis. Consistently poor growth was attained by seedlings inoculated with Sclerocystis clavispora along with Rhizobium or Rhizobium alone or by uninoculated seedlings. The combined effect of vesicular-arbuscular mycorrhiza (VAM) and Rhizobium on the cold season legumes lentil and faba bean as well as on summer legume soybean were observed in soils with low indigenous VA mycorrhizal spores. The inoculation of the plant with VA mycorrhizal fungi increased the level of mycorrhizal root infection of lentil, faba bean and soybean. The inoculation with Rhizobium had no significant effect on percent infection VA mycorrhizal infection, but VA mycorrhizal inoculation increased nodulation of the three legumes (Badr El-Din and Moawad, 1988).

## MATERIALS AND METHODS

### Culturing of Vesicular - Arbuscular Mycorrhiza

The most accessible, most abundant source of inoculum for VAM fungi is the rhizosphere of endomycorrhizal plants in the field. Spores of VAM fungi will be obtained from rhizosphere soil samples taken from 0–15 cm depth by wet sieving and decanting technique. With the aid of a dissecting microscope spores of mycorrhizal fungi will be removed from soil detritus with a microspatula or picked up by a pasteur pipette fitted with a rubber bulb and subsequently used to establish pot cultures.

### Recovery of propagules from soil

Wet sieving and decanting is a basic technique to recover propagules from the soil and to remove the clay and sand fractions of the soil while retaining spores and other similar sized soil and organic matter particles on sieves of various sizes.

### Wet sieving and decanting (Gerdemann and Nicolson 1963)

The technique is described stepwise below:

1. A volume of soil (250 ml) from the sample will be mixed in water (1000 ml) and the heavier particles will be allowed to settle for few seconds.

If the number of spores contained in various soils are to compared, the volume of soil sieved will be correlated to the dry weight of that soil sample.

2. The liquid will be poured through a coarse soil-sieve to remove large pieces of organic matter. The liquid which would pass through this sieve will be collected. The sieve will be washed in a stream of water to ensure that all small particles have passed through.

3. The particles in the liquid which have passed through the coarse sieve will be resuspended and to allow the heavier particles to settle for a few seconds.

4. This suspension will be passed through a sieve fine enough to retain the desired spores, generally 250 m. If total population is to be assessed the finest available sieve 400 m will be employed.

5. The material retained on sieve will be washed to ensure that all colloidal material has passed through the sieve.

6. Small amounts of remaining debris will be transferred to a petridish and will be examined under a dissecting microscope.

Alternatively, a nest of varied-sized sieves may be used instead of steps 2, 3, and 4. If the bottom sieve will 'clogup' either a strong stream of water or patting the side of the sieve will often unclog it.

## Identification of Genera and Species of VAM fungi

Genera and species of VAM fungi isolated will be identified on the basis of:

(A) Characters of spores

(i) Shape of spores

(ii) Attachment of hypha

(iii) Longest dimension at maturity

(B) Presence or absence of sporocarp, for example

Glomus may be sporocarpic or non-sporocarpic

Sclerocystis - Sporocarpic chlamydospores

Gigaspora - Non-sporocarpic

Acaulospora - Non-sporocarpic

Some characters for Glomus, Gigaspora Sclerocystis and Acaulospora are given (Bakshi, 1974 ) -

Glomus - Sporocarpic or non-sporocarpic, chlamydospores terminal on hyphae, oil droplet spherical, variable in size, coalesce readily, spore wall thickening extends down, one or more subtending hyphae from which germ tube emerges.

Gigaspora - Non-sporocarpic, spores (azygospores) produced terminally on a single suspensor like bulbous structure from which a slender hyphae usually projects to the spore. Oil droplet uniform, polygonal, do not coalesce, separated by a cytoplasmic net work.

Sclerocystis - Sporocarpic, chlamydospores arranged in a single layer around a central plexus of sterile hyphae. Usually sporocarps are black, brown or light brown in colour.

Acaulospora - Non-sporocarpic, resting spores borne laterally on a hyphae terminating in a vesicle.

For further identification synoptic keys (Trappe, 1982) will be consulted.

## Inoculum Production

Pure culture of VA fungi isolated and identified will be raised to maintain sufficient inoculum for experiments. The inoculum will be raised inoculating suitable host plants which will be inoculated with spores (30-50 per plant) in open pots with sterilized soil or sand. At the time of planting seeds in pots, inoculum (spores) will be layered 2-3 cm below the seed. If the seedlings are to be inoculated, the inoculum will be placed in a pot half filled with soil at the time of seedling transplants. Roots of transplanted seedlings will be put in direct contact with the inoculum, and the remainder of the soil will be added.

### Inoculum preparation for glasshouse experiments

Soil inoculum (spore, soil, hyphae, and infected mycorrhizal roots) or spore inoculum will be used depending upon the nature and objective of the experiments.

## Glasshouse Experiments

In the proposed study, effect of gaseous air pollutants ($SO_2$ and $O_3$) on the crop performance and development of VAM fungi (*Glomus* sp. and *Gigaspora* sp.) and root nodulation on gram (*Cicer arietinum* L.) and mungbean (*Vigna radiata* L.) will be studied in 30 cm clay pots in glasshouse.

Plants will be exposed to different doses of the air pollutants in exposure chambers. Following will be the pattern of treatments for experiments.

T1 Plant (control, without any treatment)

T2 Plant + Air pollutant

T3 Plant + VAM fungus

T4 Plant + *Rhizobium*

T5 Plant + VAM fungus + *Rhizobium*

T6 Plant + Air pollutant + VAM fungus + *Rhizobium*

Pots of T1, T3, T4 and T5 treatments will be kept in unpolluted air.

At the termination of experiments, the following parameters will be determined for each treatment.

Shoot length

Root length

Fresh and dry weight of shoot

Fresh and dry weight of root

Number of flowers per plant

Number of fruits per plant

Quantitative estimation of spores in soil

Intensity and percentage of VAM infection

Total numberof nodules/root system

Total number of functional nodule/root system

Chlorophyll content of leaf

Nitrogen and phosphorus content of roots

Protein content of seeds

The effect of particulate air pollutant (flyash) will be studied by artificially amending the soil with flyash obtained from the thermal power plant, Kasimpur. Soil will be amended using different proportions of flyash and plants will be grown in pots. The treatments of the experiment will be the same as in gaseous air pollutants given above and at termination the same parameters as given above will be considered.

Exposure chamber

Air pollutant exposure chambers (Standard Appliances, Varansi) will be used for the exposure of the test materials to a mixture of air and $SO_2$. The front of air pollutant exposure chamber has a full size door, and exhaust duct is provided at the top to carryout the air/gaseous mixture. The bottom is double walled and upper side has openings and the lower side is equipped with a special type of blower assembly. Fumigation controller regulates the voltage supply to blower and display

on the panel meter. The plants in pots will be kept in the gas exposure chamber and will be exposed for a desirable length of time.

Gas Generation

$SO_2$ will be generated in a generator which produces $SO_2$ gas by the action of sulphuric acid ($H_2SO_4$) on sodium sulphite ($Na_2SO_3$) under control reaction conditions. The amount of $Na_2SO_3$ and $H_2SO_4$ acid discharged from the reagent bottles mounted over the $SO_2$ generator will be determined by collecting the solution dropping through capillary tube in a graduated cylinder for sometime and expressing the rate in ml/min. On the basis of flow rate or solution feeding rate, solutions of sodium sulphite ($Na_2SO_3$) and sulphuric acid (10%) will be prepared to produce required amount of $SO_2$ gas/min, on complete reaction 1M $Na_2SO_3$ produces 1M $SO_2$ or 126 mg $Na_2SO_3$ produces 64 mg $SO_2$.

$$Na_2SO_3 + H_2SO_4 \longrightarrow SO_2 + Na_2SO_4 + H_2O$$

10% $H_2SO_4$ acid solution will be used for all the working solutions of $Na_2SO_3$.

Ozone

Ozone will be generated by subjecting dry oxygen to the action of silent electric discharge in an apparatus called Ozoniser.

$$3O_2 \rightleftharpoons 2O_3$$

## Exposure and Doses

3-4 week old seedlings will be exposed to $SO_2$ and/or $O_3$ at every alternate day for 3 h. This procedure will continue for 75 days. The concentration of gases used for exposure will be 0.1 and 0.2 ppm.

## Impact of the Air Pollutants on the Crops

For assessing the effect of the air pollutants on the pulse crops, samples from the pot experiments will be collected in polythene bags and labelled. In laboratory the root system of each plant of the samples will be thoroughly washed to remove the soil particles. Root and shoot lengths, fresh and dry weights of root and shoot will be determined for each sample by standard methods.

## Air pollution symptoms

Plants collected from the glasshouse experiments will be invariably examined closely in the laboratory for detecting symptoms on plants. If present, symptoms will be characterized and matched with the symptoms given in " Recognition of air Pollution Injury to Vegetation. A pictorial Atlas" (Eds. J. Jacobson and A.C. Hill), Air Pollution Control Association, Pittsburg, Pensylvania, 1970 and the details will be noted and photographed.

## Assessment of mycorrhizal development

### Root sample collection

Roots with fine terminal feeder roots will be exacavated from the soil in the pots of the glasshouse experiments in order to assess the mycorrhizal development. A representative sample of the entire root system will be obtained from four or five different portions of the root system and combined.

### Preserving of specimens

Individual root samples of 1-2 g (fresh weight) will be preserved in Formalin-Aceto Alcohol (FAA). The standard FAA solution will be made with 50% alcohol with V/V/V ratio of 90:5:5.

Samples collected and preserved in FAA will be used primarily for assessing the degree and intensity of mycorrhizal development and will be adequate for describing fungal morphological characteristics in the roots.

### Clearing and staining the specimens (Phillips and Hayman 1970)

1. Root specimens (fresh or preserved) will be washed with tap water and boiled in 10% KOH solution at 90°C for 45 min. or 1 h. The KOH solution will clear the host cytoplasm and nuclei and readily allows stain penetration.

2. The KOH solution will be poured off and roots will be rinsed well in a beaker using at least three complete changes of tap water or until no brown colour appear in the rinsed water.

3. Alkaline $H_2O_2$ will be used for pigmented roots i.e. to bleach the roots (Alkaline $H_2O_2$) will be made by adding 3 ml of $NH_4OH$ to 30 ml of 10% $H_2O_2$ and 567 ml of tap water.

4. The roots will be rinsed thoroughly using at least three complete changes of tap water to remove the $H_2O_2$.

5. Roots will be covered in the beaker with 1% HCl and soaked for 3-4 min and then solution will be poured off. The roots will not rinsed with water after this step because they will be acidified for proper staining.

6. Roots will be treated with 0.05% Trypan blue (0.05% Trypan blue in Lactophenol) and will be kept for one hour.

7. After removing the specimens from Trypan blue the specimens will be kept in destaining solution for overnight and then will examined under the microscope.

The standard destaining solution will consist of -

875 ml - laboratory grade acetic acid

63 ml - glycerine

63 ml - D. water

The clearing and staining techniques will remove cellular contents and make the root opaque, but VA mycorrhizal fungal structures will stain dark blue.

Assessment of colonization after clearing and staining (Giovannetti and Mosse, 1980)

The primary objective of this study will be to determine the percentage of roots colonized as well as the intensity of colonization within the roots. This will be done by slide method. Approximately 1 cm long root segments will be selected at random from a stained sample and mounted on microscopic slides in groups of 10. 30-100 root segments from each sample will be used for this method. Length of cortical colonization will be assessed (at 100X) in mm for each root segment, and will be averaged for each of the 10 segments in a group. It will be expressed as a percentage of root length colonized. This method will give an assay based on total root length taking into consideration the percentage of roots colonized, as well as the intensity of colonization.

Alternatively the slide procedure will be simplified by recording only the absence or presence of colonization in each root segment and expressing the result as a percentage of roots colonized. This method is referred to as the slide method (Giovannetti and Mosse, 1980).

## Quantitative estimation of spores from soil

The spores will be isolated from pot soil by wet sieving and decanting method. 100g of soil will be processed through this method. The suspension thus, obtained will be made up to 50 ml. The spores will be counted in 1 ml of suspension in nematode counting dish under the dissecting microscope. The final number of spores/100g of soil will be calculated accordingly.

## Plant Analysis

Analysis of plant samples from the glasshouse experiments will be done for estimating chlorophyll content in leaves and phosphorus (P) and nitrogen (N) contents of roots and leaves and protein content of seeds.

## Estimation of chlorophyll content

For chlorophyll estimation, 1g of interveinal region of the leaves will be ground in 40 ml 80% acetone with the help of mortar and pestle. The suspension will be decanted in buchner funnel having two whatman paper No.1. Then filteration will be done with the help of suction pump. The residue will be ground thrice adding with 30, 20 and 10 ml of acetone respectively. The suspension will be decanted in buchner funnel and filtered in vacuum. At last mortar and pestle will be rinsed with 80% acetone, transferred in buchner funnel and filtered in vaccum. The filtrate will be transferred in 100 ml volumetric flask and the volume will be made upto capacity. The transmittance will be read at 645, 663 and 652 nm at spectrophotometer.

The chlorophyll a, b, and total chlorophyll will be calculated accordingly by using optical density, (O.D.) (by using 1% transmittance) (Mackinney, 1941).

$$\text{Chl. a in fresh tissue} = 12.7\ (\text{O.D. } 663) - 2.69\ (\text{O.D. } 635) \times \frac{V}{100Xw}$$

$$\text{Chl. b in fresh tissue} = 22.9\ (\text{O.D. } 645) - 4.68\ (\text{O.D.}663) \times \frac{V}{100Xw}$$

$$\text{Total chl. in fresh tissue} = 20.2\ (\text{O.D.}645) + 8.02(\text{O.D.}663) \times \frac{V}{100 \times w}$$

## Estimation of N and P

For estimation of nitrogen and phosphorus root and leaf samples will be digested as given below.

## Digestion of root

100 mg of oven dried root and leaf powder will be transferred in 50 ml kjeldhal flask, then 2 ml of chemically pure $H_2SO_4$ will be added and flasks will be heated on kjeldhal assembly for about 2 h till the dense fumes has given off and the contents has turned black. Then 0.5 ml of pure 30% $H_2O_2$ will be added after 15 min of cooling. Now heating will be done again till the colour is changed into light yellow. It will be heated again for half an hour and after which flask will

be cooled for 10 min. for getting extract clear. Then 3-4 drops of 30% $H_2O_2$ will be added drop wise followed by heating for 15 min. After that digested material will be transferred in 100 ml volumetric flask with 3-4 washing and the volume will be made upto capacity. This digested material will be used for estimating N and present in the roots (Linder, 1944; Lundegardh, 1951).

Nitrogen

Prior to estimating N content present in the digested material of root and leaf standard curve will be drawn by the following procedure.

0.236 g of ammonium sulphate will be dissolved in 100 ml of solution, then 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml solution will be poured in 10 test tubes respectively. The volume will then be made upto 5 ml in each test tube by adding distilled water. A control will also be run side by side. After, that 0.5 ml Nessler's reagent will be added followed by 5 ml of distilled water. The % transmittance will be read at 525 nm from spectrophotometer on developing yellow orange colour after half an hour. Then a curve will be drawn on graph between concentration and O.D.

Estimation

10 ml of aliquot (digested root material) will be taken in 100 ml volumetric flask and 2 ml, 2.5N NaOH will be added

to neutralize the excess amount of acid present. 1 ml of 10% sodium silicate will also be added to prevent turbidity. Then volume will be made upto capacity. 5 ml of aliquot will be taken in three test tubes followed by addition of 0.5 ml of Nessler's reagent with shaking, then 10 ml volume will be made by distilled water. After waiting for 5 min the % transmittance will be read at 525 nm. Then the O.D. will help in reading the concentration from the standard curve (Linder, 1944).

Phosphorus

At first a standard curve will be prepared. Different concentrations of $KH_2PO_4$ solution ranging from 0.1 to 1 ml will be taken in 10 separate test tubes and the volume of each test tube will be maintained upto 5 ml. Then 1 ml ammonium molybdic acid and 0.4 ml of 1 amino-2-nepthol-4 sulphonic acid in each test tube will be added followed by making the volume upto 10 ml with distilled water. After half an hour % transmittance will be read at 625 nm. Then standard curve will be drawn between concentration and O.D.

Estimation

5 ml of aliquot (digested root) will be taken in three test tubes, to which 5 ml of distilled water will be added After that 1 ml of ammonium molybdic acid will be added,with shaking, followed by addition of 0.2 ml 1-amino-2-nephthol 4-sulphonic acid. The control will also run side by side.

Percentage transmittance will be read at 625 nm after half an hour. Concentration will be read from standard graph by using O.D. (Fiske and Row, 1925).

Estimation of Proteins

The protein contents of the seeds will be estimated by using the method given by Lowry et al., (1951).

Following reagents will be prepared for estimating soluble and insoluble contents of the seeds.

Reagent A - 2% sodium carbonate in 0.1N NaOH in ratio of 1:1

Reagent B - 0.5% $CuSO_4$ in 1% sodium tarterate in ratio of 1:2

Reagent C - 50 ml reagent A + 1 ml reagent B.
(Alkaline $CuSO_4$)

Reagent D - 50 ml of 2% sodium carbonate + 1 ml reagent B
(Carbonate $CuSO_4$ solution)

Reagent E - Follins reagent diluted to make 1N acid
(Diluted Follins reagent)

Root-nodule bacteria

To assess the impact on root nodulation, number of total, functional and non-functional root-nodules per plant present in the samples collected from the glasshouse experiments will

be counted. The pinkish healthy nodules will be taken as functional and others as non-functional.

Isolation of Rhizobium

After washing root in running water, a well formed healthy pinkish nodule on the tap root will be carefully cut out with a portion of root attached to the nodule. The nodule will be surface sterilized for 5 minutes in 0.1% mercuric chloride in water and repeatedly washed with sterile water to remove the chemical. The nodule will then be washed in 70% ethyl alcohol for 3 minutes followed by more washing with sterile water (Ash and Allen 1948). The nodule will be now crushed with sterile glassrod in a small aliquot of sterile water. This will be further diluted for obtaining clear and distinct colonies. Congo red yeast-extract mannitol agar medium (CRYMA) will be used for isolation. The constituents of the medium are as follows:

| | |
|---|---|
| Mannitol | 10.0 g |
| $K_2HPO_4$ | 0.5 g |
| $MgSO_4.7H_2O$ | 0.2 g |
| NaCl | 0.1 g |
| Yeast extract | 1.0 g |
| Agar Agar | 20.0 g |
| Congo red | 2.5 ml of 1% solution |
| D.Water | 1000 ml |

1 ml of the dilution will be added each petriplate containing 15 ml of CRYMA medium. The petriplates will be incubated at 30°C (±2) for one week. Distinct white, translucent, glistening, elevated colonies of Rhizobium developing on the media will be picked up and purified by reculturing.

Plant testing methods (Rao, 1975)

The ability of a particular isolate to produce nodules on a simple host legume will be determined because variation are found in strains of Rhizobium. Similarly it is also essential to know if the nodules possess efficient nitrogen fixing ability. This will be ascertained by growing plants on agar slants containing nitrogen free medium and inoculating them with the desired isolate. At regular intervals, seedlings will be fixed in 4% formalin and be examined later under a microscope for infection threads in root-hairs and emergence of nodule primordia on roots. Depending on the extent of infection, isolates may be rated for virulence.

In another set, the experiment will be continued for several weeks for obtaining the effectiveness of Rhizobium isolate by examining the dry weightof the plants. The dry weight of plant is proportional to its N-content (Erdman and Means, 1952). During the experiment the moisture content of the culture tube will be checked at the regular intervals and the nutrient solution may be changed when would be necessary.

Nitrogen free nutrient media (Jensen, 1942).

| | |
|---|---|
| $CaHPO_4$ | 1.0 g |
| $K_2HPO_4$ | 0.2 g |
| $MgSO_4.7H_2O$ | 0.2 g |
| NaCl | 0.2 g |
| $FeCl_3$ | 0.1 g |
| Agar Agar | 8.0 g |
| Distilled water | 1000 ml |
| pH | 6.8 |

## Pure culture of *Rhizobium*

Normally yeast extract mannitol agar (YMA) is used for pure culturing of *Rhizobium* (Fred *et al.* 1932).

Rothamsted collection of *Rhizobium* (RCR) have modified it slightly. The compositions of YMA is as

| | |
|---|---|
| $K_2HPO_4$ | 0.5 g |
| $MgSO_4.7H_2O$ | 0.2 g |
| NaCl | 0.1 g |
| Mannitol | 10.0 g |
| Yeast extract | 0.4 g |
| Agar Agar | 15.0 g |
| Distilled water | 1000 ml |
| pH | 6.8-7.0 |

The medium will be autoclaved at 15 lb.p.s.i. ($121^{o}C$) for 20 minutes. Then the medium will be poured in sterile

petriplates. After solidification of the medium, the tested Rhizobium isolate will be inoculated in the plates in aseptic condition. After inoculation the petriplates will be kept at 30°C (±2) in an incubator. The colonies will grow in about seven days. The culture can also be done in culture tubes containing YMA slant.

Soil based culture

For inoculation in the glasshouse experiments, the soil based culture of Rhizobium will be used for seed dressing prior to sowing.

For culturing Rhizobium in soil, the soil and compost in the ratio 1:1 will be used. 1 kg of soil compost mixture will be autoclaved and the pH will be maintained at 7 by mixing 10 gm of $CaCO_3$. After that 10 gm sugar (commercial) and 0.5 gm $K_2HPO_4$ will be added in soil compost mixture.

Then pure culture of Rhizobium on YMA will be mixed thoroughly. This mixture of Rhizobium and soil compost will be used for inoculating the seeds of the plants for experiments sowing.

Rhizobium inoculation

Rhizobium inoculation will be done prior to seed sowing. For inoculation soil based culture of Rhizobium will be used. Commercial sugar and water will be added in the soil based culture with thorough mixing. The seeds will be treated with this mixture followed by the drying in shade for about half an hour before sowing.

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